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Biological Control of Ticks with Entomopathogenic Nematodes

Principal Investigator: Dr. **Michael SAMISH,**

Grantee Institution: Kimron Veterinary Inst., ISRAEL

Collaborator: Dr. **Ellie Onyango OSIR** & Dr. **Goodwin KAAYA**

Institution: The International Center of Insect Physiology (ICIPE), KENYA

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Grant Project Officer: William H. Crane

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EXECUTIVE SUMMARY

Ticks, one of the most important pests of domestic animals, especially in LDC are now controlled mainly by acaricides. As yet no commercial anti-tick biocontrol agents exists. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) are in use commercially against certain plant pests and fleas. Experiments carried out by several scientists including Dr. Samish and Dr. Glazer indicate that some entomopathogenic nematodes also kill ticks very efficiently.

Our research aimed to create a basis for the introduction of biological control agents against ticks and then to transfer the research technology to an African country. To achieve this goal we started to search for optimal nematode-virulence, tick-susceptibility combinations as well as the efficiency of various nematode strains to kill different tick species under laboratory conditions using ticks endemic to Kenya and to Israel. This project succeeded to bring the preliminary laboratory knowledge on entomopathogenic nematode – ticks interaction to successful mini-field tick control experiments.

One of the major goals during the years was to transfer the collected information from Israel to Kenya and to study the nematode – tick interactions and the influence of various factors on the nematodes anti-tick activity under natural conditions. As Dr. Kaaya, the original principle investigator in Kenya has left his country to Namibia; efforts were devoted to transfer all the relevant information to Dr. Ellie O. Osir who replaced Dr. Kaaya.

Before the start of this project, no study on entomopathogenic nematodes – animal ectoparasites was performed in Africa. The experience gained by the African scientists can serve not only to push forward the development of an anti tick nematode based agent but also to enlarge the ability to develop such agents against other animal, human and plant arthropod pests.

A. RESEARCH OBJECTIVES

The economic losses due to ticks and tick-borne diseases (T&TBDs) are by very most in developing countries and are estimated in developing countries between US $ 13.9-18.7 billion annually.

Tick control is based, nearly solely, on chemical acaricides, which pollute the environment and raise problems of tick resistance. Biological control of most animal pest is as yet not in use. Entomopathogenic nematodes are increasingly used to control plant pests and could become a leading means for implementation in tick management.

A study of the potential of entomopathogenic nematodes for a biological control of ticks may lead to a new approach for assisting farmers mainly in developing countries in their never-ending fight against ticks.

Objective 1; Determination of the susceptibility of endemic ticks from Slovakia, Kenya and Israel to several entomopathogenic nematode strains.

Objective 2: Study of host-parasite interactions between ticks and entomopathogenic nematodes i.e. mode of nematode attraction invasion and establishment within ticks.

Objective 3: Characterization of factors in the special environment of the tick habitat, affecting nematode persistence and activity.

Objective 4: Determination of the efficacy of nematodes under natural conditions.

B. METHODS AND RESULTS

The Kenyatic partners in the project were not involved in entomopathogenic nematodes research before this project started. The visit of Prof. G. Kaaya in Israel and of Dr. I. Glazer in Kenya, the long discussions we had while meeting in conferences and via the e-mail enabled the Kenyatic team to isolate EPNs from nature, to grow nematodes in mass cultures and to use them for laboratory and field trials.

A large amount of the information gained in Israel, during this project was already published while most of the Kenyatic findings are yet unpublished (see ***Notes on Publications***).

Therefore we present in this report more details on the work done in Kenya and less on the Israeli results.

The following Ref. # indicates a reference cited in "notes on publications" in chapter E." project activity/output" of this report.

Objective 1; Determination of the susceptibility of endemic ticks from Kenya and from Israel to several entomopathogenic nematode strains.

Isolation of indigenous nematodes

Four soil samples were collected from various localities in Kenya (Rusinga Island, Kisumu, Nakuru and Nyahururu) and 4 from Arusha in Tanzania and assumingly existing nematodes were isolated. Nematodes were found in one soil sample from Kenya (Rusinga Island) and in one from Tanzania. The nematode isolated from Tanzania was found to be saprophytic whereas that from Kenya (Rusinga Island) was identified as *Steinernema carpocapsae*.

Pathogenicity tests

In this study, the pathogenicities of nematodes were assessed using fully engorged Boophilus annulatus engorged female ticks. Increasing concentrations of each isolate were applied to 5-cm Petri dishes (0.5 ml/ dish) padded with Whatman No. 1 filter paper. Five engorged ticks were placed in the Petri-dishes and incubated at 28° C (85% relative humidity in the dark). Mortality was recorded up to 14 days. The experiment was carried out in 3 replicates and each replicate had five Petri-dishes (Table 1)(ref. 6).

Table 1 – Mortality of engorged *B. annulatus* females after ten days of continuous exposure to various nematode strains in petri dishes.

Nematodes				
Species	Strain	# ticks tested	LC_{50}*	LC_{90}*
H. bacteriophora	IS-12	120	3,2	26.1
H. sp.	IS-5	80	10,0	33.1
H. bacteriophora	10C	120	82,8	94.2
H. bacteriophora	HP88	120	99,8	196.8
S. carpocapsae	S-20	120	20.2	344.1
S. carpocapsae	Mex	120	36.8	210.3
S. carpocapsae	DT	180	60.7	334.1
S. feltia	TG	120	28.2	152.5
S. feltia	Bib	100	65.4	388.5
S. riobravis	SR	120	16.8	521.8

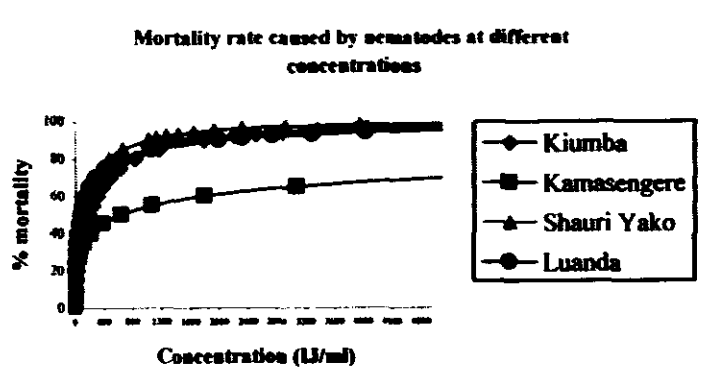


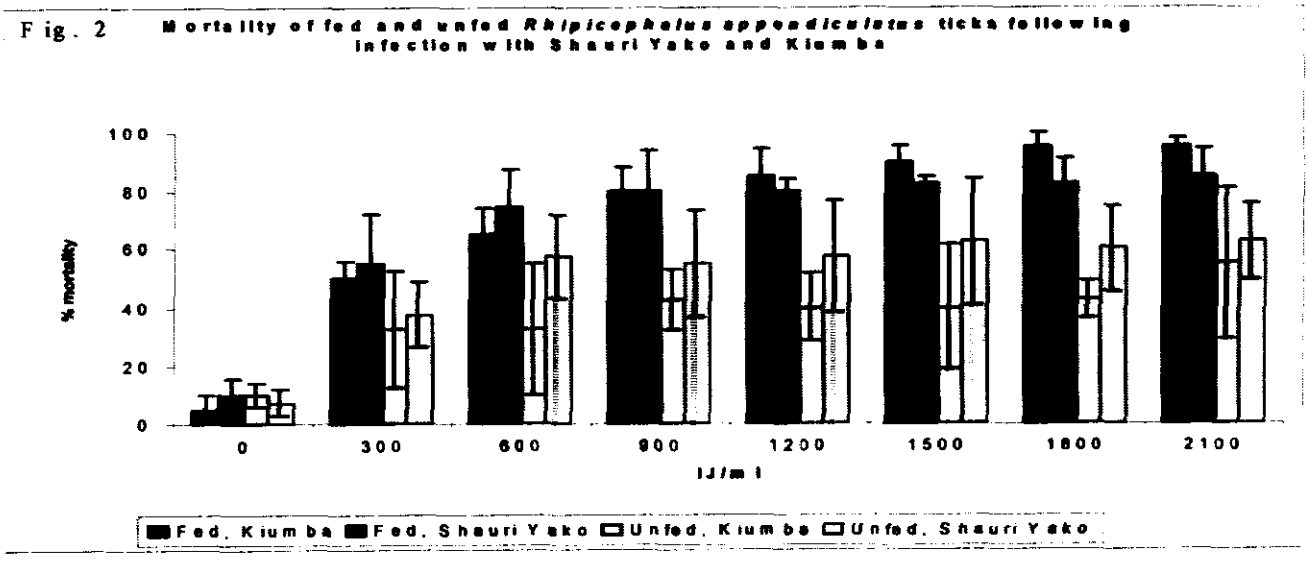
Fig. 1

Effects of various local nematode isolates on the brown ear tick, *Rhipicephalus appendiculatus*

*LC_{50} & LC_{90} – lethal concentration of nematodes needed to kill 50 or 90 percent of the ticks (expressed as # nematodes/dish).

In this study, the pathogenicities of four local nematodes were assessed using *Rhipicephalus appendiculatus* ticks. The mortality of unfed and fed adult *R. appendiculatus* ticks started one day after inoculation and stopped three days later (Fig. 1). No significant mortalities were observed on the surviving ticks for the rest of the 14-day observation period. Fed ticks started dying three days after inoculation. The LD_{50} values for Shauri Yako and Kiumba on fed ticks were 31 and 324 IJ/ml, respectively (Fig. 1, 2) (ref. 2). Similarly, the LD_{50} values on unfed ticks for Shauri Yako and Kiumba were 833 and 1140 IJ/ ml, respectively. This indicated that Shauri Yako was more virulent than Kiumba to both unfed and fed ticks. The results also showed that fed ticks were more susceptible to the nematodes than their unfed counterparts.

The mortality caused by the isolate Kamasengre, was lower than the mortality caused by the other isolates tested. Consequently, two of the active isolates (Kiumba and Shauri Yako) were selected for subsequent experiments. It should be pointed out that the mortality caused by the three isolates was comparable to those of two strains of *Steinernema caprpocapsae* (SR and Mexican) and one of Heterorhabditis (HP 88) (data not shown).



The *in vitro* effect of local Kenyatic nematode strains on unfed and engorged adult ticks (*R. appendiculatus* and *A. variegatum*).

The levels of mortality of the unfed ticks from both species were low. However, *R. appendiculatus* appeared to be slightly more susceptible (maximum mortality ~40%) compared

to *A. variegatum* (maximum mortality ~25%) (Fig. 3, 4). In previous studies, we have shown that the mortality of engorged female *R. appendiculatus* with the same nematode isolates reached ~100%, while the maximum in *A. variegatum* was ~50%. From these results it may be concluded that unfed ticks are less susceptible to nematode infection than their fed counterparts. From the results (Fig. 3), it is clear that the Kiumba, Luanda and Shauri Yako isolates caused comparably high mortalities to the engorged females.

Fig.3. **Mortality rate of unfed adult *R. appendiculatus* ticks by 2 nematode strains.**

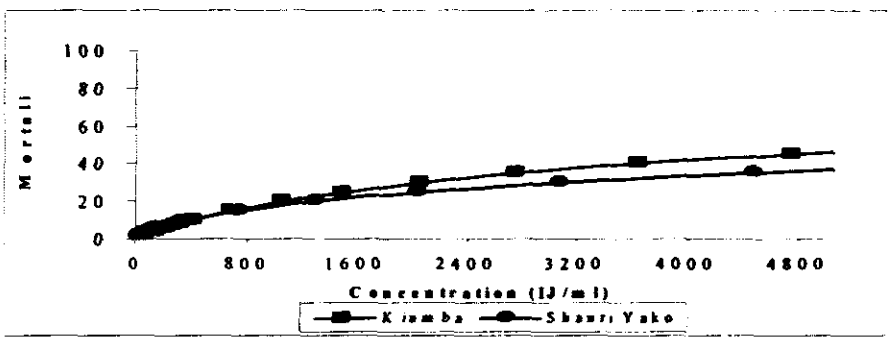
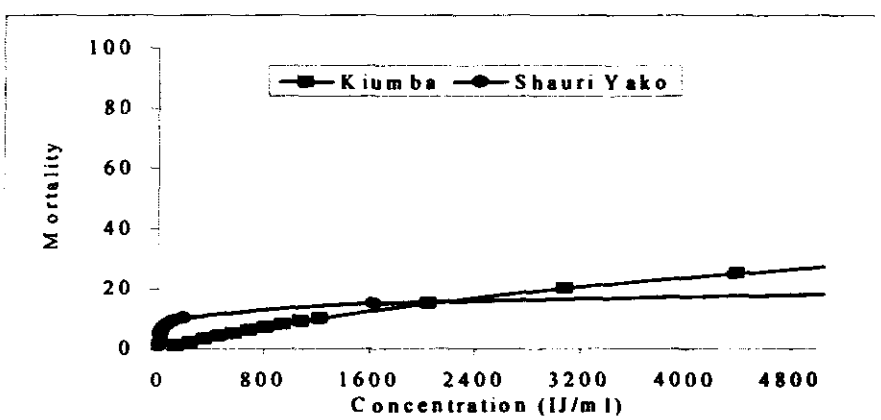


Fig.4. **Mortality rates of unfed adult *A. variegatum* ticks by 2 nematode strains.**



The susceptibility of 6 tick species at different developmental stages to strains of entomopathogenic nematodes were tested in Israel (Table 2, ref. 6). All tick species and stages were found to be susceptible. Heterorhabditids were generally more virulent to ticks than steinernematid nematodes. Strains virulent to one tick species and one stage were found, in most cases, also to be highly virulent to other tick species and stages.

The quantity of nematodes and the time required to kill 50% of the ticks (LC_{50} and LT_{50}, respectively) were lowest in trials with engorged *B. annulatus*. The LC_{50} for

engorged females was, mostly lower than for unfed adults, unfed ixodid females were killed up to 6 times more quickly than engorged ticks.

The LT_{50} for unfed *Rhipicephalus bursa* females is only 1 day in comparison to 6 days for engorged females.

Table 2 - **The effect of nematodes upon various tick species**

Ticks	Nematodes			Susceptibility of tick stages			
	# strains tested	Most virulent Species	Strain	Engorged nymphs	Unfed adults	LC_{50}/cm^2 (on day post infestation)[3]	LT_{50} (in days) (at nema/cm^2)
Boophilus annulatus	7	*H.*indicus	IS-5	+	+	<2.6 (8 d)	1.9(10/cm^2)
	9	*S. carpocapsae*	Mex			25 (6d)	2.8 (200/cm^2)
Hyalomma excavatum	5	*H.*indicus.	IS-5	+	+		2.8(255/cm^2)
Rhipicephalus bursa	5	*H.* sp.	IS-3				5.0(255/cm^2)
Rhipicephalus sanguineus	7	*S. riobrave*	HP88	+	+	>177	3.2 (255/cm^2)
Ornithodoros moubata	3	*S. carpocapsa*	DT	+			2.3(10/cm^2)
Ornithodoros tholozani	4	*S. carpocapsa*	DT	+			

Objective 2: Study of host-parasite interactions between ticks and entomopathogenic nematodes i.e. mode of nematode attraction invasion and establishment within ticks.

Attraction of Nematodes to Ticks

The mobile free living Infective Juvenile stage of nematodes are known to be attracted by insects and CO_2. Their response to ticks was not yet studied.

To determine nematode attraction by ticks we used the petri dish-agar assay. The material to be tested was placed near to the rim of a dish and nematodes were placed in its center either with the tip of a pipet or on aluminum foil to demonstrate whether volatile compounds attract/repel nematodes. In other trials the arthropod or water in which ticks have been immersed were placed directly on the agar.

All the ticks, which we tested excreted a volatile compound(s) which attracted nematodes from

the species H.b.-HP88 (Table 3). Three larvae of *G. mellonela* contained on the average more nematodes than the tested ticks. However when *B. annulatus* females or *G. mellonela* larvae were placed simultaneously with nematodes in contact with the agar their ability to attract the nematodes H.B.-HP88 could not be demonstrated (Table 4). If the female ticks were placed on the agar 2-h before the nematodes they repelled them. Water from washed cuticles of engorged *B. annulatus* females or of unfed *H. excavatum* adults also repelled the nematodes (Table 4).

Table 3 – **The influence of volatile compound(s) excreted by arthropods placed in the tip of a pipet or on aluminum foil, on the movement of H.B.-HP88 nematodes [on agar].** The arthropods and nematodes were placed simultaneously and incubated for 2 h at 26°C.

	Arthropods		Degree of attraction[1]		
	Species	Stage	Avr. x	±SE	t
On the tip of pipette	*B. annulatus*	Engorged female	70.6	8.70	2.35[2]
	G. melloneta	Larvae	91.3	4.23	9.78[2]
	H. excavatum	Unfed adults	84.8	8.68	4.01[3]
On aluminum foil	*B. annulatus*	Female[1]	96.4	96.4	12.96[3]
	G. mellonela	larvae	97.2	97.2	23.70[3]

[1]1=no attraction, no repellency; <1 repellency; >1 attraction
[2]$p<0.05$ [3]$p<0.01$

Table 4 - **The influence of arthropods (3/dish) or their cuticle extract in contact with agar upon the movement of H.B.-HP88 nematodes after incubation for 2 h at 26°C.**

[1]Areas I & II - two opposite sides of a petri dish

	Area		Arthropods h. between placing nematodes	Degree of attraction[2]		
	I[1] (test)	II[1] (control)		Avr. x	±SE	t
Live arthropods	*B. annulatus* (engorged)	Air	0	43.7	9.66	-0.65
		Air	2	28.6	5.92	-3.60[4]
	G. melonella (larvae)	Air	0	58.9	12.04	0.74
		Air	2	34.4	17.21	-0.91
cuticle wash water	*B. annulatus* (engorged)	Water	0	36.3	5.67	-2.42[3]
	H. excavatum (engorged)	Water	0	47.6	14.85	-0.16
	H. excavatum (unfed adults)	Water	0	25.8	11.27	-2.15[3]

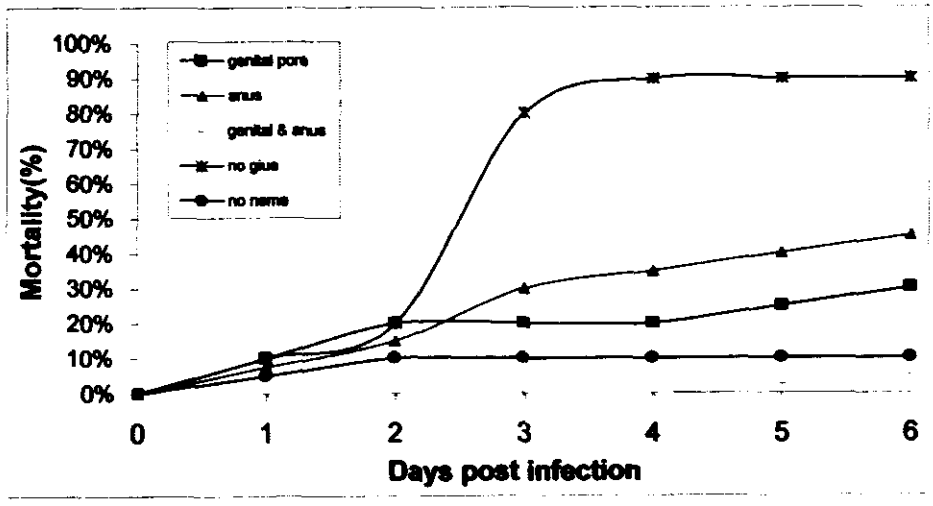
[2]no attraction no repellency; <1 repellency; >1 attraction
[3]$p<0.05$ [4]$p<0.01$

Nematode penetration and their development within the ticks. Entomopathogenic nematodes attacking insects penetrate mainly via the neutral openings of insects and release symbiotic bacteria into the haemolymph. They go through several developmental stages and have one or

more multiplication cycles within the insect cadaver before thousands of nematodes are released to the environment. In ticks such development was not observed.

One or more of the ticks natural openings were glued with 2 layers of super Glue placed in petri dishes with 5000 nematodes and their daily mortality recorded. For *B. annulatus* engorged females it was clearly demonstrated (Fig. 5, ref. 7) that the nematodes enter only via the genital pore and anus with about equal frequency. Nematodes do not seem to enter via the mouth.

Fig. 5 – Effect of gluing natural openings of engorged B. annulatus female on the ability of nematodes (*H.* sp. IS-5) to penetrate into the tick.



Amount of nematodes penetrating into *B. annulatus* engorged females was studied after exposure of the ticks to either of 6 nematode strains (5000/dish) for 36 h and dissecting them after 6 days. The average amount of recovered nematodes was recorded (Table 5). With 2 strains an average of more than 38 nematodes penetrated into each tick during 6 d of contact but less than 12 nematodes were recovered from ticks infested with 3 other strains and only 2.5 nematodes for the *S. feltia* S-20 strain. . When the ticks in a few trials were dissected immediately after 36 h of exposure the number of nematodes recovered in some cases was up to 9 times more than in ticks dissected after 6 d of exposure. Thus nematodes apparently deteriorate with time in the tick (Tables 5&6).

Table 5 - **Stages and amount of nematodes recovered and how many were alive in dissected *B. annulatus* engorged females.** Ticks exposed to nematodes (5000/dish) for 36 h and dissected 6 d after exposure.

Nematodes		# ticks	IJ & L4		Adult nematodes		
Species	Strain	dissected	# ticks	% live	# ticks	% live	Total avr. # nematodes
H. bacteriophora	IS-5	15	72	21	0	0	93.0
H. bacteriophora	IS-12	15	3.2	2.0	0	0	5.2
S. carpocapsae	DT	15	32.9	4.9	0.1	0	37.9
S. carpocapsae	Mex	15	7.2	4.6	0	0	11.8
S. feltia	S-20	15	2.5	0	0	0	2.5
S. feltia	TG	15	0.5	0	1.0	6.6	8.1

Table 6 - **The amount of nematodes recovered from *B. annulatus* engorged females. Ticks exposed to nematodes (5000/dish) for 6 d before dissection.**

Nematodes				
Species	Strain	Diameter (μm)	# ticks dissected	Avr. # nematodes recovered/tick
H. bacteriophora	IS-5	23	30	141
H. bacteriophora	IS-12	23	30	36
S. carpocapsae	S-20	25	15	70
S. carpocapsae	DT	25	20	36
S. feltia	TG	26	25	115
S. glaseri	SG	43	25	130

The large difference in amount of nematodes/tick in tables 2 and 3 probably indicate that many nematode strains succeed to enter the ticks also after the first 36 hours of contact.

The development of nematodes within ticks

Engorged *B. annulatus* ticks were kept in Petri dishes with 5,000 nematodes/dish for 5-6 days, washed and dissected. The amount of nematodes in ticks was recorded according to whether they were live or dead and according to their developmental stage. Five nematode strains from 2 species were compared. The ability of the nematode strains to invade the ticks varied conspicuously with the maximum average of 235 nematodes for the H.b.-IS5 strain and as little as 6.9 for the H.b. Megidis. The amount of nematodes from each strain invading ticks has no connection to its success to start developing within the ticks. None of the nematodes developed further than to the stage of larval number 4. nearly a quarter (23.2%) of H.b.-Megidis developed into its L_4 stage during the 6 days while among the other tested strains much fewer succeeded to develop into the L_4 stage (Table 6, ref.7).

The effect of tick organs upon the development of nematodes *in vitro*

Various aliquots from ground tick organs and other compounds of symbiotic bacteria and infective juveniles were placed into wells with a nematode growth medium. On days 2,4,6(?) live and dead nematodes as well as the stage of their development in each well were recorded. On day 2 all nematodes placed in the wells were observed but no development could be seen. On day 4 fewer nematodes could be found while some started to develop. On day 6 there was no significant difference between wells with the various additives.

The two phases of symbiotic bacteria in ticks (*in vivo*)

The symbiotic bacteria of entomopathogenic nematodes (*Xenorhabdus nematophila, Photorabdus luminescens*) are known to appear in two phases called I and II. Only phase I shows up in insects, the normal host of nematodes. Phase I in contrast to phase II is known to support the development of the nematodes within it's host and to produce antibiotics helping to get a monoculture of the symbiotic bacteria within the host.

Three days after infecting engorged *Boophilus annulatus* ticks or the greater wax moth (*Galleria mellonella)* they were dissected and their various organs were ground. Aliquots were seeded into Petri dishes with Targitol – 7 agar. After 2 days of incubation the amount of bacterial colonies of phase I was recorded and compared with those of phase II. In all cases of infection with *G. mellonella* we found only phase I but in ticks infected with H.b.-IS5 we found only phase II. In tick infected with S.c.-Mex there was often a mixture of the 2 phases (ref. 7).

Objective 3: Characterization of environmental factors, in the special environment of tick habitat, affecting nematode persistence and activity.

Viability of nematodes in different soil types or cattle manure-under lab. conditions

In this study, two local nematode isolates (Shauri Yako and Kiumba) were used. These isolates have previously been shown to be pathogenic to ticks (*Rhipicephalus appendiculatus* and *Amblyomma variegatum*). Four different soil substrates (sand, loam, red soil and manure) were used. The nematode isolates (1200 infective juveniles/ ml) were each mixed with 250 ml of the different soil types or with pure cattle manure in 330ml plastic cups and incubated for either 24 or 48 h at 28° C. After incubation, *Galleria* larvae (5 per container) were introduced to each plastic container and incubated for 72 h at 28° C. The experiment was carried out in 3 replicates. Mortality of *Galleria* larvae in cages buried to a depth of ~3cm in the soil, were recorded.

The mortality of *Galleria* larvae is shown in Figs. 6, 7. For both nematodes isolates, loam soil, red soil and manure did not have any effect on the nematodes as evidenced by 100% infection and mortality of *Galleria* larvae. The nematodes were slightly affected by sandy soil as shown by a reduction in *Galleria* infection of ~20%. The reason for this loss of activity is not clear since a special effort was made during the experiment to ensure that all the soil types should remain moist. Further studies will be needed to clarify this point.

Fig. 6 **Efficacy of nematodes (Shauri Yako isolate) in different soil types 1&2 d post application**

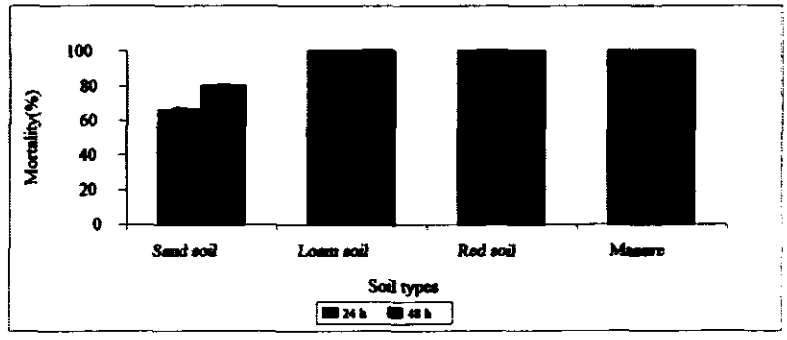
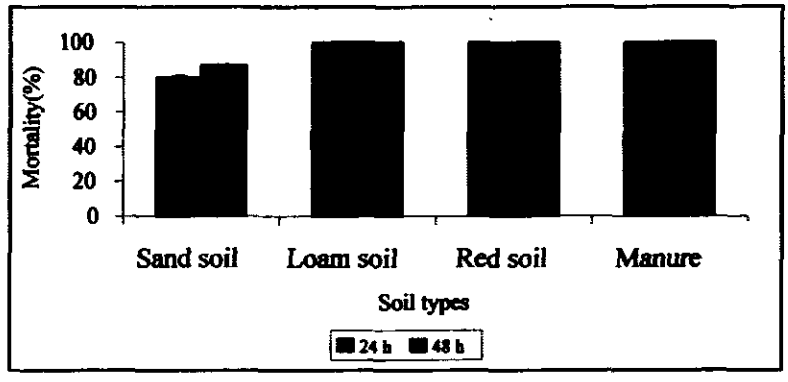


Fig. 7. **Efficacy of nematodes (Kiumba isolate) in different soil types 1&2 d post 4.**

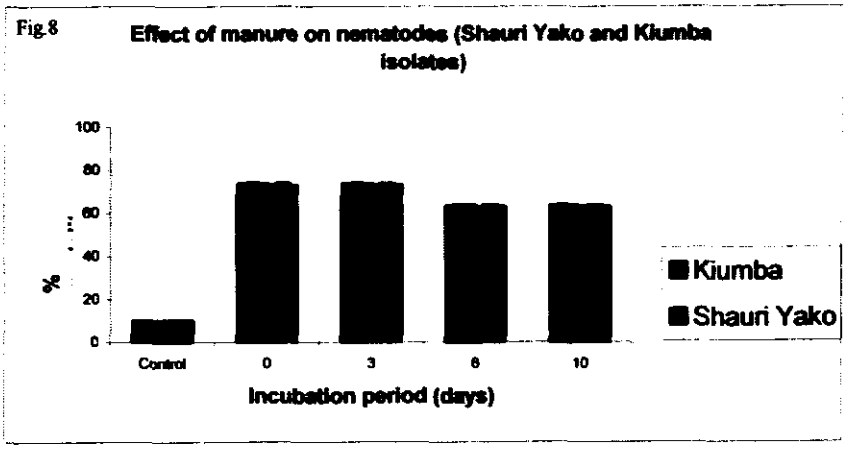


Survival of nematodes in manure

Prior to the semi-field experiments, it was important to assess whether manure has any effects on the nematode isolates intended for use in subsequent studies. In the experiment, plastic containers were filled with 0.85L, 10 cm deep, mixture 50:50 of manure and red Nairobi soil. The nematodes, Shauri Yako isolate, was mixed with the manure at 1200 IJ/ml into the manure samples. After 0, 3, 6 and 10 days under shade outdoors, 10 fully engorged *R. appendiculatus* ticks were introduced into each container. Mortalities were recorded from the 8th up to the 14th day after introduction of the ticks. The ticks were also dissected to detect the presence of nematodes. The experiment was carried out in 3 replicates.

From the results (Fig.8), incubating the nematodes in manure with soil for up to 10 days did not affect their viability and ability to infect ticks. Mortality levels were generally high. Dissection of ticks that died showed the presence of nematodes proving that mortality was due to the nematodes. Manure takes up moisture rather fast and it was therefore necessary to keep it moist by adding a few drops of water every day. Since the experiment was carried out under a cattle shed, we did not cover the containers with grass. Similar results were observed using another virulent isolate, Kiumba (Fig. 8).

Fig. 8

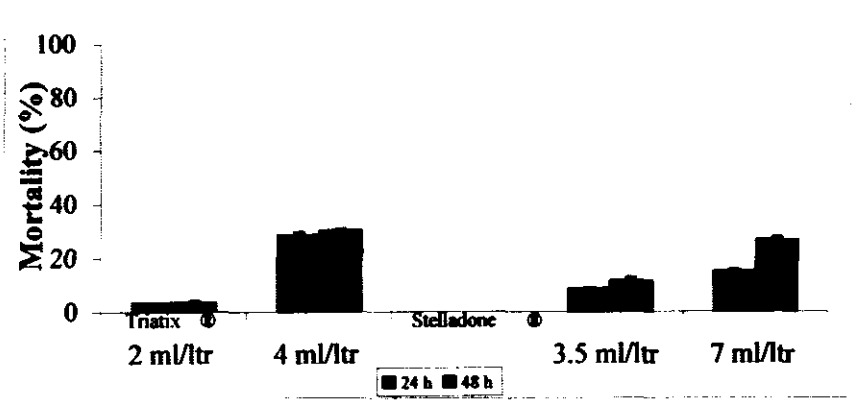


Effect of commercial acaricides on nematodes

In an integrated tick management strategy involving the use of nematodes, they will possibly come into contact with acaricides that are also used by farmers. This experiment was carried out to assess the compatibility between commonly used commercial acaricides and nematodes. Two acaricides, namely, Triatix^{R} (Amitraz, Welcome) and Steladone^{R} (Chlorfenvinphos, Ciba-Giegy), were selected for the experiment. They were added to Petri dishes (55 mm diameter) containing 20 nematodes (Shauri Yako isolate) at concentrations of 2 and 4 ml/l (Triatix^{R})(0.25g/liter) and 3.5 and 7 ml/l (Steladone^{R}) (5.25g/liter) resp. and incubated for 24 and 48 h at 28° C. The 2 and 3.5 ml respectively are the recommended concentration for spray on cattle. For each compound, the lower concentration is that recommended for tick control by the manufacturers. The viability of the nematodes was then determined using a microscope. The experiment was carried out in 3 replicates.

At concentrations recommended for tick control by the manufacturers, both acaricides caused only little mortality of the nematodes (Fig. 2). At twice the recommended concentrations, mortality levels of ~20% were observed in both cases. The duration of exposure did not seem to affect the levels of mortality. It is concluded that the nematodes are compatible with these commonly used acaricides.

Fig. 9. **Effects of acaricides on death of nematodes (Shauri Yako strain)**



We repeated these experiments and included two other commonly used acaricides. In the experiment, the compounds were Bovitraz[r] (Amitraz, Bayer) and Supona[R] (Fort Dodge). Each Petri-dish (55 mm diameter) contained 20 nematodes (Shauri Yako isolate) and incubated at 28° C for 5, 10, 24 and 48 h. The viability of the nematodes was determined microscopically.

All compounds tested caused negligible mortality at concentrations recommended by the manufacturers (Fig. 10,11). However, increased mortality was observed when the concentrations were doubled. Even so, the values were generally less than 30%.

Fig.10

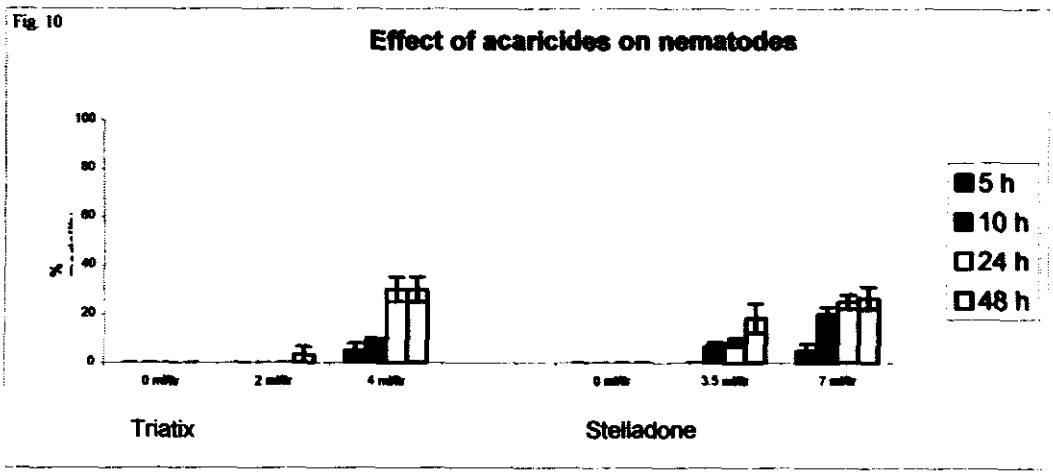
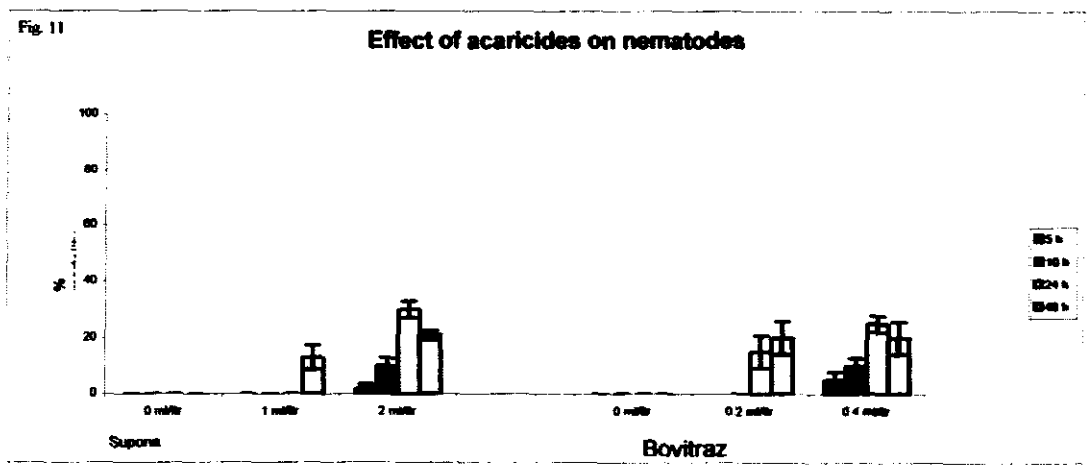


Fig.11



<u>The effect of antibiotics upon the survival of nematodes *in vitro*</u>

Five antibiotics from different groups which are used commonly during the care of farm animals and expelled via the urine of treated animals were tested at their approximate maximum concentration in urine. Ten ml of the solution to be tested (water served as control)

were poured into 5 cm ϕ petri dishes with about 1500 live nematodes and kept at 26°C. At known intervals 0.5 ml aliquots were removed and the percentage of dead nematodes recorded. Each combination was carried out in 4 replicates. Among the 5 antibiotics tested the *H. sp.* IS-5 was generally more resistant than S.c.-DT. Erythromycin seems to be the least harmful. In the first 24 h a maximum reduction in nematode survival was due to Gentamycine which reduced S.C.-DT survival by 44% in comparison to the control. The influence of the other 4 antibiotics was far lower (Table 7).

Table 7 - **The effect of various antibiotics upon the survival of 2 nematode strains in petri**

Nematode		*Heterorhabditis* sp. 'IS-5'					
Compound*		Control	Erythro.	Genta.	Strep.	Sulfa	Tetra.
mg/ml		0	50	250	250	500	50
% Survival after	24 h.	94.2	93.0	88.6	90.8	83.0	87.9
	48 h.	86.1	87.9	82.6	85.6	80.7	78.6
	72 h.	85.0	81.7	64.9	68.5	68.1	67.6
Nematode		*Steinernema carpocapsae* 'DT'					
% Survival after	24 h.	88.2	85.2	58.0	80.0	74.5	76.8
	48 h.	81.8	78.1	39.8	66.4	65.8	62.6
	72 h.	73.4	65.2	34.2	67.1	59.5	63.7

dishes at 26°C (average of 4 replica).

* Erythro. = Erythromycin; Genta. = Gentamycin; Strep. = Steptomycin; Sulfadimidine = Sulfa; Tetra. = Tetramycin.

Outdoor Experiments

Effect of nematodes on ticks under field conditions

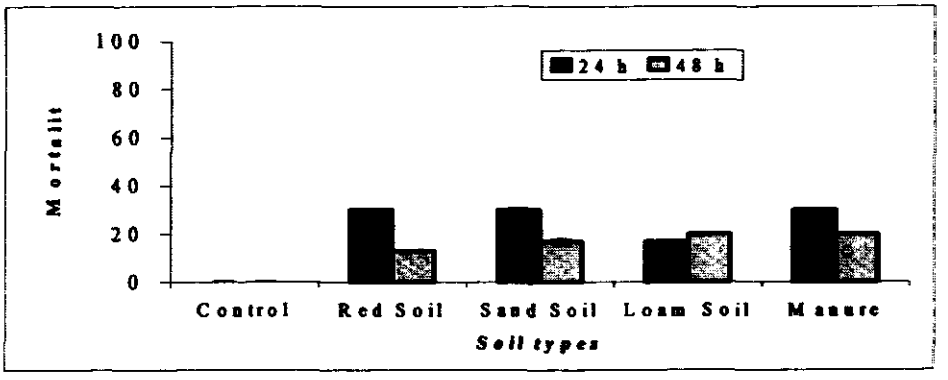
Experiment A - This experiment was carried out to assess the viability of nematodes in different soil types (red soil, loam soil, sand and cattle manure) under field conditions. Plastic containers (d-10.5 cm and h-20 cm) were filled with the different soil types. The nematodes (Shauri Yako isolate, 200ml water/container, 1.4 nematodes/cm^2) were sprayed on the soil. To prevent rapid drying of the soils, they were covered with grass. After 24 and 48 h, ten engorged *R. appendiculatus* female ticks were introduced in each container. Mortalities were recorded from the 8th to the 14th day after introduction of the ticks. The ticks were also dissected to record the presence of nematodes. Soil temperatures were monitored for the entire duration of the experiment. The experiment was carried out in 3 replicates.

By the 14th day, the mortality of the ticks was generally low (~20 - 30%) in the different soil types (Fig. 12). No mortality was recorded in the control experiment. Dissection of the ticks

that died showed the presence of nematodes suggesting that the nematodes caused the mortality. The low mortality rates in this experiment may have been caused by the fact that only few ticks burrowed into the soil and thus did not come into contact with the nematodes. Despite the grass that was used to cover the soil, most of the soils dried somewhat due to the unusually dry weather conditions prevailing in Kenya and the ticks could not easily burrow into dried soil.

Other experiments on the influence of soil types on the nematodes efficiency was summarized elsewhere (Ref. 7, 8).

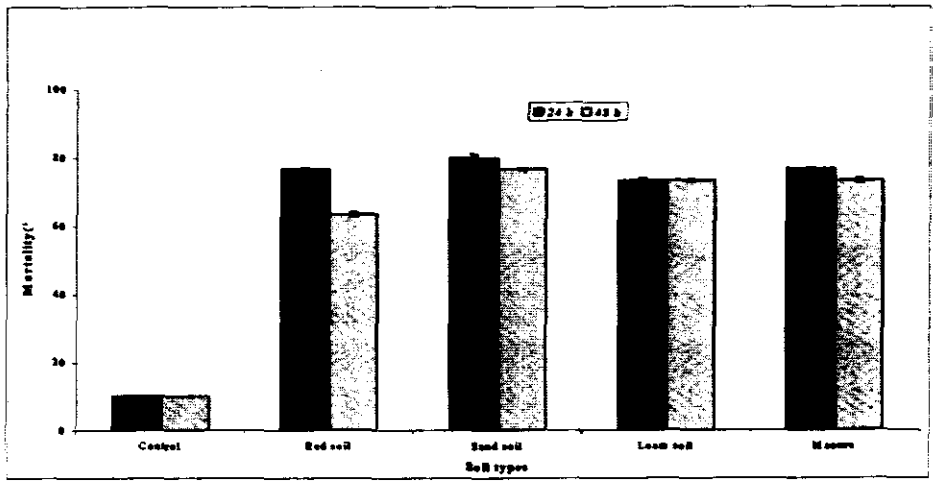
Fig. 12 **Mortality of engorged *R. appendiculatus* female ticks placed on intact soil sprayed with nematodes (Shauri Yako isolate) under field conditions.** Ticks applied 1 and 2 d after nematodes.



Experiment B - This study was carried out essentially as Experiment A. Since it was observed (Exp.A) that by the time of application, the soil surface had already hardened (Fig. 12). The top of each soil sample was slightly loosened with a spoon enabling the ticks to behave naturally borrowing about 3 cm into the soil. Experiment B and the ticks were simply dropped on to the surfaces of the soil samples

Compared to Experiment A, high mortality was recorded when the surfaces of the soil samples has been gently loosened prior to introduction of the ticks (Fig. 13). It was observed that almost all the ticks were able to burrow into the soil. This presumably made it easier for them to come into contact with the nematodes and hence the high mortalities. In addition, it rained heavily on the 4 and 5th days after the nematodes were introduced. This may have also increased the time that the nematodes remained active in the soil.

Fig. 13 **Mortality of engorged *R. appendiculatus* female ticks placed on loosened soil surface sprayed with nematodes (Shauri Yako isolate) under field conditions.** Ticks applied 1 and 2 d after nematodes.



The efficiency of nematode strains against ticks in buckets under simulated field conditions

As an intermediate between laboratory and field experiments we used 10 liter buckets filled with soil with nematodes and ticks. They were placed in a temperature and humidity controlled greenhouse so as to study the anti-tick efficiency of nematodes.

Nematode strains and concentration - The 9 tested nematode strains differed markedly in their effect upon tick mortality. Their relative virulence was not parallel to that obtained in our previous petri dish laboratory test. At a concentration of 50 nematodes per cm^2 one efficient nematode strain caused 100% tick mortality. Similar levels are being recommended for nematode agents used commercially against insects (Table 8,Ref. 1, 7, 8).

Virulence of nematode strains with time - Ticks were placed into 10 l buckets 0 or 3 days after they had been sprayed with nematodes and tick mortality was recorded periodically. The loss in nematode virulence in sandy soil varied during 3 days between 3.2-31.3% depending on nematode strain.

Table 8 - **Mortality (%) of *B. annulatus* engorged females (n=80) exposed to nematodes (200IJ/cm^2) in 10 l buckets with sandy soil (10 d post infestation).**

Ticks added days after spraying nematodes	Nematode strain			Control
	H.b.-HP88	S.c.-Mex	S.f.-TG	No. nematode
0	87.5	100	63.2	0
3	66.6	68.7	60	0

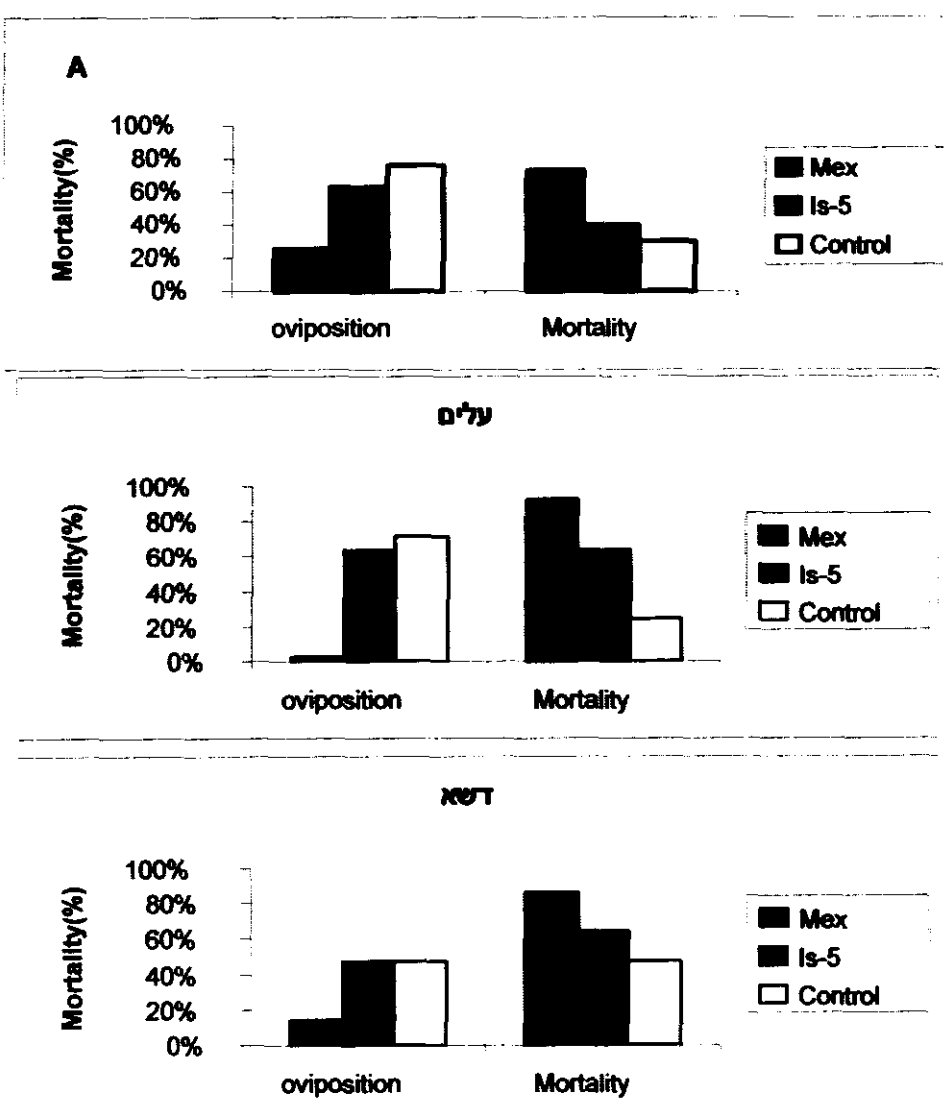
Objective 4: Determination of the efficacy of nematodes under natural conditions.

Outdoor experiments- effect of soil cover

Recently a field experiment consisted of three areas each divided into 9 mini 50x50 cm plots. Each plot was enclosed with a smooth 10 cm wall so as to prevent an escape of the ticks.

The 3 sandy soil areas were: I uncovered (only a few leaves and bulbs), II loam - each plot fully covered with 2 layers of under tree cover first small pieces of semi rotted leaves and above it a dry whole leaf cover. The plots were sprayed with 200 nematodes/cm^2 and after 2 h 50 engorged female ticks per plot were scattered on top. Humidity of air and soil was recorded daily. The nematode *Steinernema carpocapsae* Mex strain (9 plots, 450 females) was more virulent than the *Heterorhabditis bacteriophora* IS-5 strain under these experimental conditions (Fig. 14). With the 2 types of soil cover the nematodes were for more efficient in killing the ticks and less ticks started to lay eggs than in the uncovered soil plots. Nematodes succeeded to kill up to 92% of the ticks and only 2.3% of the surviving ticks started to lay eggs.

Fig. 14 **Mortality of ticks and % of females starting to lay eggs in outdoor plots with two nematode strains.** The plots were (A) uncovered (B) covered with leaves or (C) covered with loam. All values 21 days after start of trial.



1) Mini-plots

Twenty seven sheet metal squares (50x50 10 cm high) were pressed 2 cm deep into a loamy sand area. The squares were covered with a light metal net to prevent birds and rodents form preying on ticks. The 27 squares were divided into 3 equal areas to obtain triplicates. A computerized irrigation system supplied 4.2 liter water/meter during each irrigation.

The temperature and humidity of the air and soil were recorded during the entire experimental period. The pre-irrigated plots were sprayed with 200 nematodes/cm^2 and 2 h later 30-50 fully engorged *Boophilus annulatus* females were scattered in each square plot. Three weeks later the collected ticks were divided into 3 groups: those which have died before laying eggs (dead & swollen) those which died after laying eggs (dead & shrunken) and live female ticks.

a. Effect of soil cover (partially reported in our second year report). The influence of 3 types of soil covers on the anti-tick activity of 2 nematode strains (*Heterorhabditis bacteriophora* Is-5 strain and *Steinernema carpocapsae* Mexican strain) were examined. The plots were covered either with: (1) only several stones and a few leaves, (2) dense green grass, (3) a leaf layer of old semi-decayed foliage below a fluffy leaf layer imitating a soil cover in forests.

The average soil temperature at 10-12mm depth was measured under the loam, under the leaf layer and in the uncovered soil. There seems no doubt that differences in temperature and humidity affected the activity and survival of the nematodes (Fig.14).

In all parameters tested the nematode S.c. – Mex. was far more virulent to ticks under field conditions than the H.b.-IS5 nematodes.

In the control plots (no nematodes) the loam cover supplied a good ecological niche for the survival and egg laying of the ticks significantly better than the leaf cover the uncovered soil plots were a poor shelter for the ticks (Fig.14).

Both nematode species tested showed a far better anti-tick activity in the two types of soil which were covered than in the uncovered plots. The difference between the influence of loam versus leaf cover on the H.b.-IS5 was not significant while for the S.c.-Mex. strain, the leaf layer seemed to supply better conditions for their anti-tick activity (Fig.14).

b. Effect of shade The uncovered soil and the leaf layer plots were kept either (1) uncovered or (2) covered with a black plastic net providing 50% shade or (3) 90% shade. The degree of shade does not seem to influence the mortality of ticks in the plots without nematodes (control) neither had much influence on the soil temperature (Ref. 7). However

increasing the shade on plots with S.c.-Mex. nematodes increased the total percentage of dead ticks as well as those which died before laying eggs and decreased the amount of ticks laying eggs in comparison to the uncovered plots.

The improvement in nematode anti-tick activity was parallel to the reduction of the soil temperature.

c. Effect of irrigation This experiment was performed during the rainless summer season. The plots were irrigated once a day before application of the nematodes and/or ticks as well as (1) one every other day (2) every day or (3) twice a day. Each irrigation 4.2 liter/m^2. The irrigation intervals did not influence the survival and activity of ticks in plots without nematodes with either a leaf layer or without a soil cover (Fig.14).

In plots without cover the higher the irrigation the more efficient the nematodes activity. However, leaf layers reduced the influence of the irrigation on nematodes activity (Ref. 7, 18).

2) Small plots

The effect of nematodes on ticks in semi-field conditions

This preliminary study was conducted to assess whether nematodes can be deployed for tick control under semi-field conditions. The nematode isolate selected, namely, Shauri Yako, had been previously shown to be effective against *Amblyomma variegatum*, the species selected for the following experiment.

The first experiment was carried out in the open and the aim was to assess the value of nematodes for controlling ticks off-host in the vegetation. Small plots (10 ft x 10 ft) with grass were prepared. An area of about 2 ft around each plot was cleared of grass. A small plastic barrier was placed around each plot in the cleared space to prevent ants and other insects from entering. Each plots was seeded with 150 adult unfed ticks (*A. variegatum*). In order to assess survival, the ticks in each plot were determined using a pheromone trapping method. This method uses a trap containing both the attracting aggregation attachment pheromone (AAAP) and carbon dioxide. This device has already been shown to be capable of attracting ticks up to 5 m. However, high temperatures have been shown to reduce the attraction of ticks.

Table 9: **Recovery of ticks in control plots (before onset of the dry season)**

Number of days post-seeding	Mean % number of ticks recovered
0	89.5 ± 2.6a
5	87.1 ± 2.0a
10	88.3 ± 1.3a
15	89 ± 2.5a
20	31.8 + 1.1b
21	18.2 + 2.2b

Means in the same column followed by the same letter are not significantly different at the 5% level based on the SNK test.

Table 10: **Recovery of ticks in plots during the dry season**

Number of days post-seeding	Mean % number of ticks recovered
0	22.2 + 1.1a
5	19.5 + 0.5a
10	9.2 + 0.2b
15	-
20	-
21	-

Means within the same column followed by the same letter are not significantly different at the 5% level based on the SNK test.

In this experiment, the recovery of ticks in the control plot was very good up to 15 days (mean % values > 80). From 15 days onwards, we started to observe less recoveries perhaps due to natural mortality or to predation by ants. Unfortunately, just before we initiated seeding of the experimental plots, an unusually dry season set in. In the weeks and months that followed, very few ticks were recovered from the vegetation and by day 10, no live ticks were found (Table 9,10). The conclusion we drew was that most of the ticks succumbed to desiccation.

B. *IMPACT OF PROJECT:*

The laboratories in ICIPE, Kenya have previously not worked with entomopathogenic nematodes. Their success in starting to perform experiments with such nematodes is no doubt thankfully due to this AID/CDR project.

During the first year a very good collaboration between the 3 collaborating scientists was established. Dr. G. Kaaya visited the laboratories of Dr. I. Glazer and Dr. M. Samish learned a long list of methods and performed mini trials (Attachment 1). The knowledge gained during this visit served as the basic platform on which all further experiments in Kenya were performed. Entomopathogenic nematodes were transferred from the Israeli collection to Kenya.

Dr. Glazer visited ICIPE, Kenya during March 1999 and gave advice on how to optimize the experiments. In addition e-mail messages were exchanged between the participants in order to discuss the various steps of the experiments.

During the second year Dr. G. Kaaya left ICIPE, Kenya and was replaced by Dr. Osir Ellie Onyango. Dr. Osir who was orally assisted by Dr. Kaaya and by surface and

electronic mail by Dr. Samish and Dr. Glazer so as to become acquainted with the subject. The effort devoted during the first year to teach Dr. Kaaya will probably be valuable since he is now employed by the University of Namibia where he plans to carry out research in order to advance the development of tick biocontrol strategies.

During the last 2 years the very good collaboration continued between the 3 collaborating scientists. Dr. Osir Ellie Onyango, Dr. Samish and Dr. Glazer in designing and concluding from the various experiments.

The employment of entomopathogenic nematodes for the control of ticks should help markedly to improve the present serious problems caused by ticks in most of Africa including Kenya due mainly to the present use of chemical acaricides which result in an increasing build-up of resistance of the ticks and in environmental pollution.
During the last 2 years our efforts were devoted primarily towards transferring the laboratory success of killing ticks with nematodes to field conditions which had an impressive success. This is a highly important step towards the future commercial use of nematodes against ticks for use by farmers.
The International Center of Insect Physiology & Ecology (ICIPE), Kenya research institute serves as a center for large parts of Africa. Even though it investigates ways to control arthropods it has previously never considered in the past a potential use of entomopathogenic nematodes for the biocontrol of arthropods. The commercial use of such nematodes for the control of insect plant pest is increasing in most developed countries. A successful effort made in this project to transfer the knowledge concerning such nematodes to ICIPE should not only help in the control of ticks but also towards the development of nematode based agent for the control of plant pests. The involvement of more scientists, such as Dr. Osir and Dr. Kaaya, to the biocontrol of veterinary pests will be of importance when this future research results will be introduced.

D. *PROJECT ACTIVITY/OUTPUT*

1) Meetings

The results of this project were presented in several scientific meetings, see *"Notes on Publications"*. During 1999 Dr. Kaaya (Kenya) and Dr. Samish (Israel) participated in

the *"5th Biennial Conf. Soc. Trop. Vet. Med.*, (FL., USA)" and presented results gained while performing this project. There they found a good occasion to discuss and to plan further experiments in detail.

2) Training

During the first year Dr. G. Kaaya spent 20 days at the laboratories of Dr. I. Glazer and Dr. M. Samish to learn a long list of methods and to perform different mini trials (see Attachment A).

***3)* Note on Publications**

The present project enabled us to publish the following publications:

1) Samish, M., E. Alekseev & I.Glazer. (1999). Efficacy of entomopathogenic nematode strains against *Boophilus annulatus* (Say) (Arachnida: Ixodida) engorged females under semi-natural conditions. *J. Med. Entomol.*, 36:727-732.

2) Kaaya, G.P., Glazer, I. & Samish, M. (2000). Laboratory evaluation of pathogenicity of entomopathogenic nematodes to African tick species. *Ann. N.Y. Acad. Sci.* 916:303-308.

3) Samish, M. (2000) Biocontrol of ticks. *Ann. N.Y. Acad. Sci.*, 916: 172-178.

4) Samish, M., E.A. Alekseev & I. Glazer (2000). Biocontrol of ticks by entomopathogenic nematodes: Research Update. *Ann. N.Y. Acad. Sci.*, 916:589-594.

5) Glazer, I., E. Alekseev & M. Samish (2001). Factors affecting the virulence of entomopathogenic nematodes to engorged *Boophilus annulatus* ticks. *J. Parasitology* 87:808-812.

6) Samish, M. & I. Glazer, (2001). Entomopathogenic nematodes for the biocontrol of ticks. *Trends in Parasitology* (previously *Parasitology Today*) 17: 368-371.

7) Zangi, G. (2003). Tick control by Entomopathogenic Nematode and Fungi. M.Sc. Thesis.

8) Alekseev, E., I. Glazer & M. Samish, (In preparation)(Draft attached)

Effect of Soil Types on the Activity of Entomopathogenic Nematodes Against *Boophilus annulatus* Ticks.

9) Samish, M., E. Alekseev & I. Glazer, (In preparation) The affected of manure on Entomopathogenic nematodes.

Parts of the results obtained from this grant were presented in different scientific meetings and it's abstracts were published in:

10) Samish, M. (1999). Biocontrol of ticks. *5th Biennial Conf. Soc. Trop. Vet. Med.*, (FL., USA), p. 122.

11) Samish, M. (1999). Can entomopathogenic nematodes be used for the control of ticks? *5th Biennial Conf. Soc. Trop. Vet. Med.*, (FL., USA), p. 123.

12) Samish, M., Alekseev, E.A. & Glazer, I. (1999). Entomopathogenic nematodes controlling ticks under simulated field conditions. *3rd Int. Conf. on Ticks and Tick Borne Pathogens: into the 21st century*, (High Tatra Mountains, Slovakia), p. 90.

13) Samish, M. & Glazer, I. (1999). The potential use of nematodes and fungi to control ticks.
Int. Symp. on Biological Control Agents in Crop and Animal Protection, (Swansea, UK), 1 p.

14) Kaaya, G.P., Samish, M. and Glazer, I. (1999). Pathogenicity of entomopathogenic nematodes to African ticks species. *5th Biennial Conf. Soc. Trop. Vet. Med.*, (FL., US), p. 85.

15) Samish, M., G. Gindin, E. Alekseev & I. Glazer (2000). Control of ticks with entomopathogenic nematodes and fungi. *21st Int. Cong. Entomol. (Foz do Iguassu, Brazil*, p. 539.

16) Samish, M., G. Gindin, E. Alekseev & I. Glazer (2000). Microbial control of ticks. Israel Journal of Veterinary Medicine, 55(3):115.

17) Samish, M., G. Gindin, E. Alekseev & I. Glazer (2001). The control of ticks by entomopathogenic nematodes and fungi. *The 18th Conference of the Entomological Society of Israel. Phytoparasitica* 29: 69.

18) Samish, M., Alekseev, E.A. & Glazer, I. Entomopathogenic nematodes – some factors affecting their virulence to ticks (2001). 8th European meeting of IOBC/WPRS working group – insect pathogens and insect parasitic nematodes. Greece.p71.

19) Ellie O. Osir, ; Michael Samish , Peter Arama and Mark Kimondo (2001). Use of Entomopathogenic Nematodes for the Management of Livectock Ticks. First Regional Conference of the Kenya Society of Microbiology, Nairobi, Kenya.

20) Samish, M. (2003). Entomopathogenic nematodes for the control of veterinary pests. The 3ed Int. Symp. on Entomopathogenic Nematodes and their Bacteria, Ohio, USA. p.31

21) Samish, M. and Glazer, I. (2003). EPNs against veterinary pests. Int. Workshop on Entomopathogenic Nematodes and their Bacterial Symbionts, Eilat, Israel. Abstract, p. 23.

E. PROJECT PRODUCTIVITY

The project accomplished all of the proposed goals.

F. FUTURE WORK

This project succeeded to develop the preliminary laboratory findings that entomopathogenic nematodes can kill specific Israeli tick species and stage into success to kill several African and Israeli tick species in semi field experiments. Now we have the encouraging background information to start field experiments while developing a suitable formulation.

Attachment A

Visiting Scientist – Study Plan

Dr. G. Kaaya (ICIPE, Kenya) – Visit to the Kimron Veterinary Inst. and the ARO, The Volcani Center, Israel - 20.10.98 - 9.11.98

Methods studied:

1. Isolation of nematodes from soil	2. Growing *Galleria mellonella*
3. Counting nematodes	4. Infecting ticks with nematodes
5. Growing nematodes in *Galleria*	6. Ticks – nematodes in bucket and soil test
7. Tick – nematode petri dish test	8. Recognizing ticks killed by nematodes
9. Recognizing live/dead infective juveniles	10. Nematode pathogenicity tests (humidity, temp. resistance)
11. Recognizing nematode stages	12. Dissecting ticks
	13. Isolation & culture of symbiotic bacteria

Mini projects:

1. Killing ticks by nematodes (quantitative, qualitative rate a: in petri dishes; b in buckets and soil.
2. *Galleria* nematodes: various nematode strains development of nematode and dose response at various temperatures.

DRAFT

Submitted to: **Biological Control** (~~29 April~~10 May 04)

Effect of Soil Types on the Activity of Entomopathogenic Nematodes Against *Boophilus annulatus* Ticks

Eugine Alekseev[1], Itamar Glazer[2]*, and Michael Samish[1]

[1]Kimron Veterinary Inst. Bet-Dagan, POBox 12, 50250, Israel

[2]Dept. of Nematology, ARO, The Volcani Center, Bet-Dagan, 50250, Israel

Running title: Activity of Nematode in Soil Against Ticks

*Corresponding author:

Dr. Itamar Glazer

Department of Nematology,

ARO, The Volcani Center,

Bet-Dagan, 50250, Israel

Fax: 972-3-9604835

Tel: 972-3-9683298

E MAIL: glazeri@netvision.net.il

ABSTRACT

Michael, you said that you have the abstract somewhere

Key words: Biocontrol, *Boophilus annulatus*, Entomopathogenic nematodes, *Heterorhabditidae steinernematidae*, Ixodidae, Ticks.

INTRODUCTION

The third stage infective juveniles (IJs) of entomopathogenic nematodes (EPNs)can locate and penetrate insect hosts through natural body openings and release symbiotic bacteria within the intestines of the host (Bedding & Molyneux, 1982). Only the infective stage of these nematodes is able to survive outside the nematode-killed insects. The nematodes feed upon the rapidly-multiplying bacteria and debris, and subsequently mature, mate, and produce two or more generations within the insect cadaver before emerging into the environment as IJs in search of a new host. It has been used to control many insect pests (Kaya and Gaugler, 1993; Georgis and Manweiler, 1994; Martin, 1997).

Numerous studies have demonstrated wide variations in the virulence of EPNs, as shown by the rate and degree of mortality of their hosts (Caroli, *et al.* 1996; Poinar, 1979). However, most studies were conducted in the laboratory, demonstrating differences in infectivity under extreme conditions, in which the target pests is exposed to the nematode on an artificial substrate such as filter paper or agar (Glazer and Lewis, 1998).

Studies during the last decade showed that EPNs are also pathogenic to ticks (Mauleon, et al., 1993; Samish and Glazer, 1992, 2001; Samish *et al.* 1999*b*; Zhioua *et al.*, 1995). Out of 16 ixodid tick species from 6 genera and three argasid species from two genera tested, only 1 species was not susceptible to these nematodes (Samish et al,. 2001). Similarly to insects, large differences in susceptibility to nematodes was recorded among engorged females of the various tick species in Petri-dish assays (Samish et al,., 2001). Fully engorged argasid and ixodid female ticks were generally very sensitive to EPNs infection (8-11). Furthermore, the 42 nematode strains from different steinernematid and heterorhabditid species tested in the laboratory for their anti-tick activity, varied in the degree of virulence (Samish et al,.2001). Heterorhabditids were generally more virulent to ticks than steinernematid nematodes (Hassanain, 1999; Kaaya, 1999; Glazer et al, 2001). Strains virulent to one tick species were mostly found, to be also highly virulent to other tick species (Hassanain, 1999; Kaaya, 1999; Samish et al,., 2001).

Under field conditions it was found that steinernematide strains, particularaly the Mexican and DT strain of *S. carpocapsae* are more effective against ticks than the heterorhabditid strains (Samish et al., 1999).

It was noted that nematode behavior and environmental factors may have an important influalce on nematode activity against the tick. The soil is the natural habitat of EPNs. It has been shown that in the soil environment some nematode species search for their host near to the soil surface (e.g. *Steinernema carpocapsae*) (Moyle and Kaya, 1981; Kaya and Gaugler 1993) adopting an "ambusher" searching approach, whereas others are adapted to a "cruisier" searching approach deeper in the soil (e.g. *Heterorhabditis bacteriophora*) (Lewis, 2001). Such behavior is not evident in petri dish infectivity bioassays. Nevertheless, soil texture, chemistry and moisture have a profound effect upon the EPNs activity and persistance (Kaya, 1990). Consideration of the effect of soil components on nematode activity are a pre-requisite for the sucessful use of EPNs against new target pests.

In the present study we investigated the effect of soil type and characteristics on nematode behavior and effectiveness against ticks. For this purpose we tested nematode strains from both Steinernema and Heterorhabditis genera that showed, in previous laboratory tests the highest virulence against ticks. Engorged female of *Boophilus annulatus* were the target host.

MATERIALS AND METHODS

Ticks and nematodes. Boophilus annulatus ticks were collected in 1984 from cattle in Israel and since fed every 2 months on Friesian calves. The off-host stages were incubated in the dark at 26°C, 80% RH. The engorged female ticks were tested for nematode susceptibility within 24 h after repletion.

For the origin of the nematode strains (Glazer et al J. parasitol.87, 2001)tested in this study is reported in Table. 1. The nematodes were reared at 25°C in the last instar of the

Wax Moth Galleria mellonella (L.), according to the method of Kaya and Stock (1997). After 7-14 d of storage at 8°C, they were left to acclimatize at 21°-23°C for 24 h.

Bioassay system. The experimental arena in the present study consisted of 10 l cylinderic plastic buckets (400 cm^2 soil surface and 21 cm deep) filled with soil up to a two third depth. Unless indicated otherwise sandy loam soil (table 2) was used. The soil was oven dried (70°C for 24 h) and than moistened to 13% (w/v) or to different levels of field capacity if mentioned. Stones and a layer of eucalyptus leaves were placed on the soil to create a habitat similar to natural conditions in the field. Infective juveniles ($200/cm^2$)were applied on the soil surface in a 40 ml water per bucket. One to two hours after the nematode application, 20 engorged *B. annulatus* females were placed on the soil surface of each bucket. The buckets were maintained in a climat-controlled glass house, at 26±2°C and 80±3% RH, under natural illumination. All treatments consisted of 4 replicates (buckets) and each experiment was repeated 3 times.

Tick mortality, (no movement of legs when stretching them artificially) was recorded daily for 14 d. Distribution of different nematodes strains in soil cores was detected three days after their application as follows: A plastic cylinder (2.5 cm diam.) was forced vertically into the soil. The column of soil in the cylinder was slowly pushed out and cut into 1 cm slices. Slices (approx. 5 cm^3) from different depths (0-1, 1-2, 2-3 or 5-6 cm) were put on a 60 mesh screen which was placed above a 1 cm layer of water in 9 cm diam. Petri dishes. After 24 h of incubation in room conditions the number of nematodes in the water was checked Three soil samples were taken from each bucket. Using this experimental systems the following aspects were determined:

a.

*Statistical analysis.*Data on the virulence was analyzed using the General Linear Model procedure and Turkey's multiple range test (Anonymous, 1996). Mortality data expressed as percentages were transformed into the arcsine of a square root and analyzed using a

contingency table and the χ^2 test. The lethal dosages required to kill 50% or 90% of the population (LD_{50}, LD_{90}) were calculated using Prohibit Analysis (Anonymous, 1996). All comparisons were made at an 0.05 level of significance.

RESULTS

The distribution of nematodes in different soil depths varied consiberably according to the various nematode strains (Fig. 1). For all strains very few nematodes were found at the depth of 6 cm. Among the steinernematids, most IJs were found at the depth of 1-2 cm. The majority (> 80%) of IJs of *S. carpocapsae* Mexican and DT strains as well as IJs of *S. riobrave* TX were found at the upper soil layer. Whereas more IJs of *S. feltiae* and *S. carpocapsae* S-20 were found also of 2 and 3 cm deep (Fig. 1). Among the 3 heterorhabditid strains tested here low numbers (>3) of nematodes were recovered from all depths in the HP88 strain of *H. bacteriophora* and the IS-12 strain of *Heterorhabditis* sp. Infective juveniles of the IS-5 strain of *Heterorhabditis* sp. were found at similarconcentration to those of steinernematids. This strain had the most IJs at a depth of 6 cm of all strains tested (Fig. 1).

Mortality of engorged females of the *B. annulatus* was highest with heterorhabditid strains IS-12 and IS-5 (> 85%). To some lower extent high mortality was observd with *H. bacteriophora* HP88 and *S. riobrave* (Fig. 2). Under the same conditions the mortality caused by all other steinernematid strains was lower than 50% (Fig. 2).

The presence and activity of IJs of *S. carpocapsae* DT at the soil upper layer (1 cm depth) was affected by soil type. The highest number of IJs was recorded in pots containing hamra soil (Fig. 3). Comparatively, 50-70% less nematodes were found in the lighter soils, sand sea and kurkar. Very few nematodes were recoved from the heavy type soils, shalhabim and beitnir (Fig. 3). Almost 100% mortality of engorged *B. annulatus* females was recorded in pots containing Hamra, Sea sand and kurkar. Whereas only 40-45% mortality was found in pots containing shalhabim and beitnir soils (Fig. 3).

A drastic reduction of nematode numbers in the upper soil layer(70-80%) was recorded in Hamra soil at moisture a of 80-40% as compared with soil moistend to full field capacity (Fig. 4). Despite the reduction in nematode numbers, soil moisture of 40% and

above was sufficient to warnt?? high efficacy (> 85% mortality) of the IJs among engorged female ticks (Fig. 4).

DISCUSSION

Most of the tested entomopathogenic nematode strains displayed a typical dispersal profile in the soil according to their search behavior characteristics. The species *S. carpocapsae* and *S. riobrave* were categorized as "ambushers" (Lewis, 2001) had as expected a relatively high number of nematodes on the upper soil surface (Fig. 1). Heterorhabditid strains which are characterized as "cruisers" in their foraging behavior (Lewis, 2001) almost disappeared from the soil profile even as low as 6 cm. The IJs of *S. feltiae,* which are known to be in between?????, and the S-20 strain of *S. carpocapsae*, which is genetically selected for its attraction to CO_2 sources (Lewis, 2001), were found in higher numbers in deeper soil layers (Fig. 1).

Ticks typically live on the soil surface and prefer the humid environments of the the upper soil layer, under stones or when covered with leaf litter which also favour entomopathogenic nematodes. Although one would expect that efficacy of nematode would be related to the number of IJs found on the surface no such relation was found in the present study. Contrarily, the IS-12 strain of the *Heterorhabditis* sp. was recovered only in low numbers had the highest killing effect, whereas most steinernematid strains tested here persist in relatively abundance on the surface had a lower efficacy (Fig. 1 and 2). This can be explained either by the assumption that once a host is placed on the surface the nematodes from the deeper soil layer emerge to infect it. Another possibility is that, although nematodes were applied on the soil surface uniformly, some "pockets" with higher numbers remained under protected micro-niches such as under stones and leaf litter, which is also a favorable environment for the engorged female ticks. Noteworthy, the present efficacy data are in agreement with the preformance of the various strains in our laboratory assays (Samish and Glazer, 2001; Samish *et al.*, 2000), indicating that these laboratory tests may be

effective for identification of highly effective nematode strains against different tick species.

All nematodes are aquatic organisms and need a film of water surrounding their body in order to move (Norton, 1978). Dry conditions affect nematode motility and survival adversely. The data obtained in the present study, in regard to the effect of soil texture and moisture strongly indicate that moisture affected the presence and activity of nematodes at the upper soil layer:

Soil texture- The reduction of nematode numbers in light soil can be explained by the fact that water evaporation is more rapid in these soils than in Hamra (Fig. 4). The IJs either die due to the fast desiccation or migrate to deeper soil layers which retain sufficient humidity. The drastic decline in nematodes in the upper layer of the heavy soils (Shalabim and Beitnir) is attributed to the fact that the IJs could not penetrate radily into these soil types and remain exposed to rapid desiccation. This decline also reduced nematode efficacy. Nevertheless, tick mortality was also high at low soil moisture (Fig. 4). This can be explained by the above theory, i.e. attraction of nematodes to the host from deeper soil layers or the higher concentration of nematode in protected niches.

Soil moisture- The results show that even when the number of nematodes found on the upper layer drastically declined as a result of reduced moisture (Fig 3), ticks mortality remained high.

The results obtained here strongly imply the need for careful consideration of the environmental conditions before EPNs can be effectively applied against ticks on soil surface. Nonetheless, some nematode strains were highly effective even under less favorable conditions for their activity and persistence in the soil. Our data support the assumption that EPNs can be used as an effective biological control agent of ticks.

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Table 1- Type of soils studied, their source and composition.

Soil type	Location	Code name	pH	Sand (%)	Silt (%)	Clay (%)	Texture	גיד כללי (%)	Field capacity (%)
Sandy loam (C-Horizon Rhodoxeralf)	Bet Dagan	SL	8.4	88	0	11	חול סייני	1.4	28
Marine sand	Beach	MS	8.3	90	0	9	חול	5.5	28
Calcareous sand stone	Nes Ziona	CSS	8.3	83	5	11	חול סייני	26.8	28
Haploxeroll soil (Mollichorizon)	Shalabim	HS	7.6	23	27	49	חרסית	15.9	72
Haplargid soil	Bet Nir	HSC	7.8	19	27	53	חרסית	18.2	68
Calcareous xerochrep	Tel Goded	CX	6.8	45	23	31	חול חרסיתי חולי	27.7	70

Ledgends to Figures

Fig. 1 - Distribution of different nematode strains in sandy loam soil 3 d after application of 200 IJs/cm^2 in 10 l cylinderic plastic buckets (400 cm^2 soil surface and 21 cm deep). Nematode name abriviations: Hb= *Heterorhabditis bacteriophora*; H= *Heterorhabditis* sp.; Sc= *Steinernema carpocapsae*; Sf= *S. feltiae*; Sr= *S. riobrave*.

Fig 2 – Mortality of engorged *Boophilus annulatus* females after (????? how many days????) exposure to different nematode strains applied at concentration of 200 IJs/cm^2 in 10 l cylinderic plastic buckets (400 cm^2 soil surface and 21 cm deep). Nematode name abriviations: Hb= *Heterorhabditis bacteriophora*; H= *Heterorhabditis* sp.; Sc= *Steinernema carpocapsae*; Sf= *S. feltiae*; Sr= *S. riobrave*.

I think we can combine fig 1 & 2. Makes more sense!!!! Call it Fig 1 a & b

Fig 3 - Effect of different soil types (see table 1) on mortality of engorged *Boophilus annulatus* females and the amount of nematodes in the upper 1cm of soil 3 d post application at concentration of 200 IJs/cm^2 in 10 l cylinderic plastic buckets (400 cm^2 soil surface and 21 cm deep). The ticks were introduced to the arena 3???d after spraying nematodes. ()

Fig 4 - Effect of moisture on the amount of nematodes and on mortality of engorged *Boophilus annulatus* females and the amount of nematodes in the upper 1cm of soil 3 d post application at concentration of 200 IJs/cm^2 in 10 l cylinderic plastic buckets (400 cm^2 soil surface and 21 cm deep). The ticks were introduced to the arena 3???d after spraying nematodes.

Fig 3

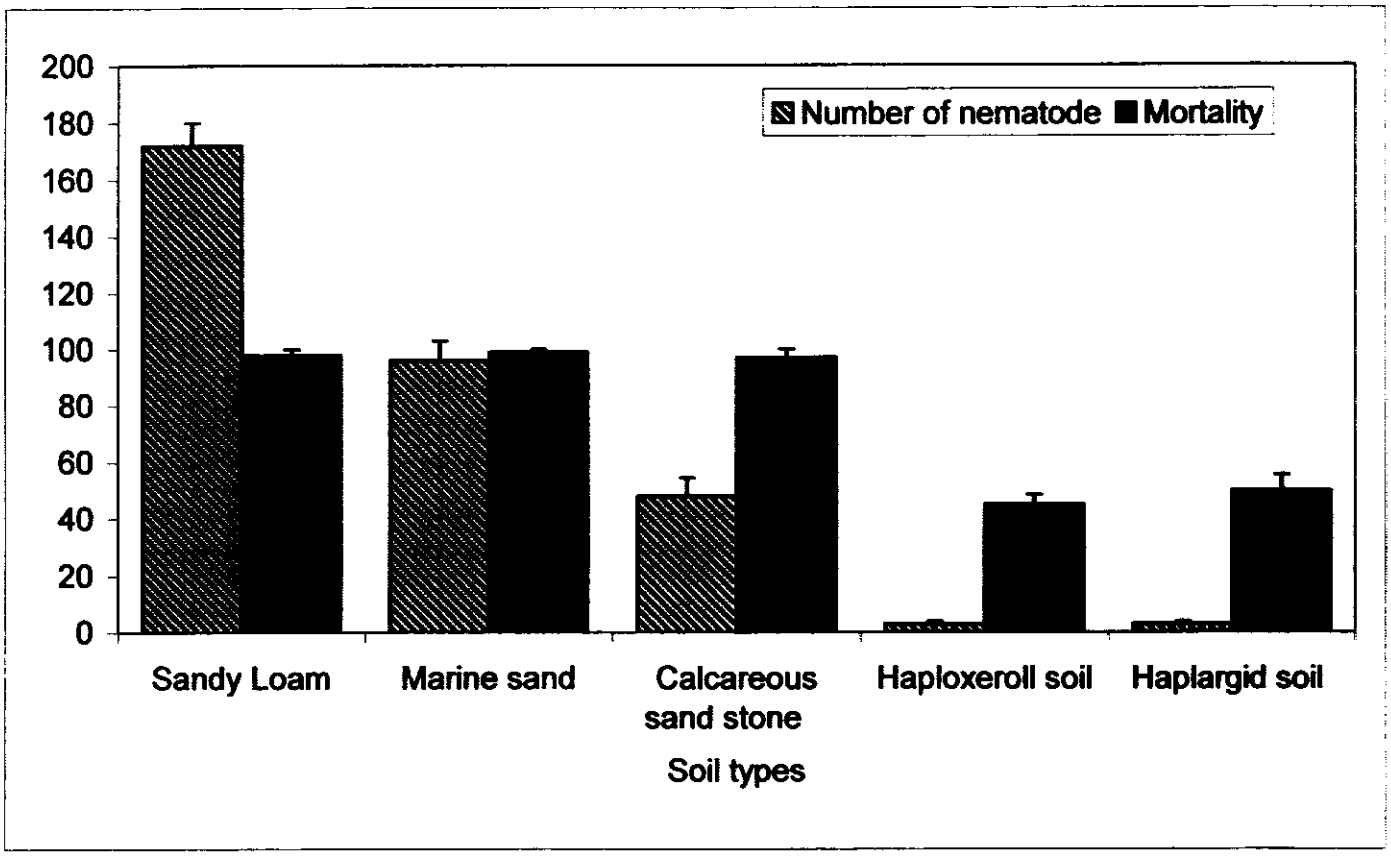
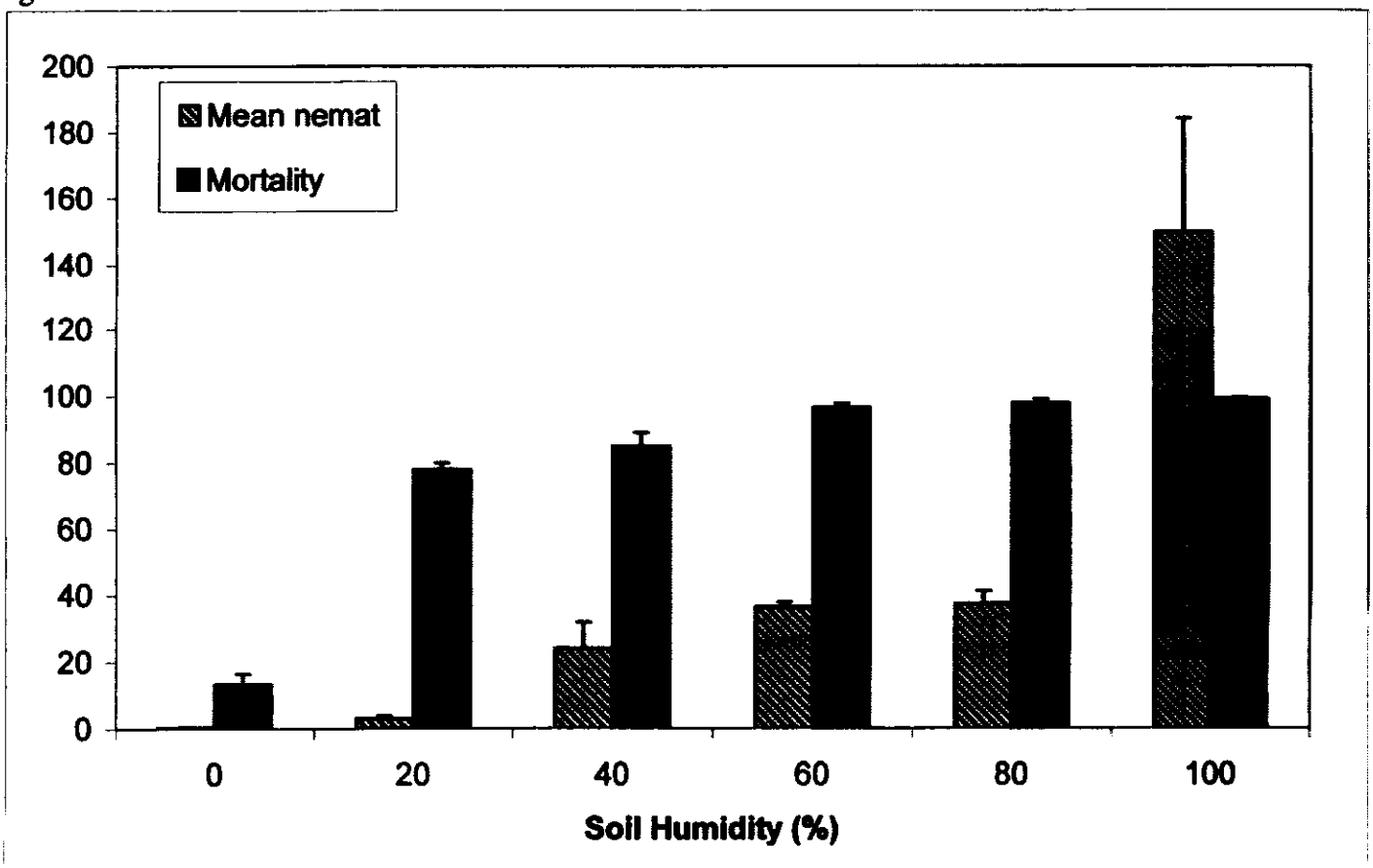

200
180
160
140
120
100
80
60
40
20
0
Number of nematode
Mortality
Sandy Loam
Marine sand
Calcareous sand stone
Haploxeroll soil
Haplargid soil
Soil types

Fig. 4


200
180
160
140
120
100
80
60
40
20
0
Mean nemat
Mortality
0
20
40
60
80
100
Soil Humidity (%)